

iGEM TU/e 2014

Biomedical Engineering

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Preparative steps

Preparation of culture media, agar plates, antibiotics and glycerol

Table of contents

Title Preparative steps	1	Preparation of Culture Media	3
	1.1	Luria-Bertani (LB) medium (300 mL)	3
	1.2	2YT medium (for protein expression) (100 mL)	3
	2	Preparation of Agar Plates	3
	2.1	LB-agar (500 mL; ~25 plates)	3
	2.2	Pouring agar plates	3
	3	Preparation Antibiotics	4
	3.1	Kanamycin (5 mL)	4
	3.2	Chloramphenicol (for protein expression) (4 mL)	4
	4	Preparation of Glycerol	4

1 Preparation of Culture Media

1.1 Luria-Bertani (LB) medium (300 mL)

For 1L

- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- Add 1L H₂O

1.2 2YT medium (for protein expression) (100 mL)

For 1L

- 16 g peptone
- 5 g NaCl
- 10 g yeast extract
- Add 1L H₂O

2 Preparation of Agar Plates

2.1 LB-agar (500 mL; ~25 plates)

For 1L

- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- 15 g bacto-agar
- Add 1L H₂O

Both media and agar need to be autoclaved (sterilization).

2.2 Pouring agar plates

- After autoclaving the LB-agar at 121 °C for 20 minutes, let the agar cool down to ~50 °C (autoclave can be opened at 90 °C). Make sure the agar does not start solidifying
- Add kanamycin and /or chloramphenicol to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri dishes until the bottom is well covered. Work near the Bunsen burner flame
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench
- Transfer the plates to a bag, in which they should be placed upside down
- Store the plates in the fridge (4 °C)

3 Preparation Antibiotics

3.1 Kanamycin (5 mL)

30 mg/mL stock solution in H₂O. Final concentration in cultures and LB-agar: 30 µg/mL
Filter stock solution using 0.2 µm filter.

3.2 Chloramphenicol (for protein expression) (4 mL)

25 mg/mL stock solution in ethanol. Final concentration in cultures and LB-agar: 25 µg/mL
Filter stock solution using 0.2 µm filter.

4 Preparation of Glycerol

- Obtain 100% Glycerol.
- Create a 50% v/v solution with H₂O.
- 1 mL of 100% glycerol weighs 1.26 g thus for 100 mL of 50% glycerol, weigh out 63 g of 100% glycerol and add 50 mL of H₂O.
- Mix well.
- Take 500 µL (in het OGO Het Lab in protocol is dit 300 µL) of desired bacterial broth to form stock.
- Add 500 µL of 50% glycerol stock to the broth.
- Mix well.
- Store at -80°C
- When required for use, defrost stock and streak small amount on agar plate to obtain colonies.